CRYPTIC SUBSPECIATION IN DROSOPHILA BELONGING TO THE SUBGENUS SOPHOPHORA[1]

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INTRODUCTION

It is generally believed that subspecies often represent incipient species and that, therefore, knowledge of many details of the speciation process might be expected to be revealed by studies of subspecies in genetically well-known organisms—especially subspecies of the sort designated "borderline" by Patterson and Dobzhansky (1945) and characterized by development of almost, but not quite, enough reproductive isolation to be considered good species.

The genetically best-known species belong to the genus Drosophila. Yet, among the 274 Drosophila named in Patterson and Stone's (1952) recent classic on evolution in this genus, there are only 15 forms that fall in their seven groups of subspecies, all belonging to the subgenus Drosophila.

In the subgenus Sophophora there is the *willistoni* group which includes the very interesting *willistoni* sibling group that has been described in detail by Dobzhansky (1946) and Burla *et al.* (1949). *D. tropicalis* Burla and da Cunha belongs to this sibling group. In general the centers of geographic distribution of these sibling species are in Brazil.

Collections of the *willistoni* sibling species taken by the writer, first in Cuba and later throughout the Greater Antilles, included a previously undescribed subspecies of *D. tropicalis*. The present paper describes this subspecies, which is named *D. tropicalis cubana*, and compares it morphologically and genetically with the South American subspecies which, following taxonomic convention, is now designated *D. tropicalis tropicalis*.

FORMAL DESCRIPTION OF NEW SUBSPECIES

Drosophila tropicalis cubana, subspecies nova

♂. Arista with 10–13 branches. Antennae yellow, third segment somewhat darker and with rather long dense pilosity. Front over $\frac{1}{3}$ width of head, wider above; dull yellow. Anterior orbital about $\frac{9}{10}$ and middle about $\frac{2}{5}$ posterior. Two or three prominent oral bristles, second nearly as long as first. Carina short, gradually falling off below; rounded; neither sulcate nor nose-like. Face and cheeks pale yellow; latter's greatest width about $\frac{1}{8}$ greatest diameter of eyes. Eyes bright red with short light colored pile.

Achrosticals in 6 rows; no prescutellars. Anterior scutellars parallel. Mesonotum and scutellum tannish yellow, slightly shining. Pleurae and legs pale yellow; apical bristles on first and second tibiae, preapicals on all three. Sternoindex about 0.33.

Abdomen yellow, slightly shining; each segment with an uninterrupted diffuse dark brown apical band fading out laterally.

Wings clear. Costal index about 1.9; fourth vein index about 1.9; 5x index about 1.7; 4c index about 1.3. Dorsal bristle at apex of first costal section stouter, but not longer, than ventral one. Third costal section with heavy bristles on basal ½.

Length body 2.14 mm. (living specimen); wing 1.95 mm.

♀. Length body 2.46 mm.; wing 2.22 mm.

Internal characters of imagines.

Testes pale yellow, with about 3 inner and 2 outer coils or gyres; vasa deferentia not fused, or distal ends partially fused for distance up to four times diameter of single vas deferens.

Spermathecae subspherical, width being somewhat greater than height, chitinized, dark brown; ventral receptacle a flat spiral with about 7 coils, bent into W-shaped plate lying against vagina.

Two anterior and two posterior Malpighian tubes, ends free.

Other characteristics, relationship, and distribution.

Eggs.——Two filaments, apical ⅖ much expanded, thin, spatula-like; about ⅘ length of egg.

Puparium.——Yellowish tan. Each spiracle with about 9-11 branches on very short stalk.

Chromosomes.——Metaphase plate with one pair of rods and two pairs of V's.

Relationship.——Belongs to the willistoni group of the subgenus Sophophora.

Distribution.——Collected in the Greater Antilles: Soledad Central, Cienfuegos, Cuba; Baracoa, Cuba; Constant Spring, Jamaica; Port-au-Prince, Haiti; Ciudad Trujillo, Dominican Republic; Río Piedras, Puerto Rico; and Caribbean National Forest, Puerto Rico.

Holotype male.——Holotype and paratypes descendants of a single female collected at Soledad Central, September 1950; holotype and some paratypes to be deposited in American Museum of Natural History; some paratypes deposited in collection of Department of Zoology and Entomology, University of Tennessee, Knoxville.

Note.——Females of *D. tropicalis tropicalis* and *D. tropicalis cubana,* on the one hand, differ from those of the other sibling species in that the latter have about five more coils in their ventral receptacles and also have less chitinized spermathecae.

MATERIALS

At the outset of this study nine strains of *D. tropicalis cubana* from Soledad Central, Cienfuegos, Cuba, a strain of *D. equinoxialis* from Tefé, Amazonas, Brazil, and one strain each of *D. tropicalis tropicalis, D. willistoni,* and *D. paulistorum* from Belém, Pará, Brazil, were available. Later strains of *D. tropicalis cubana* from Baracoa Cuba, Constant Spring, Jamaica, Port-au-Prince, Haiti, Ciudad Trujillo, Dominican Republic, and Río Piedras, Puerto Rico, and, most recently, a strain of *D. tropicalis tropicalis* from Içana, Amazonas, Brazil, became available.

MORPHOLOGICAL DIFFERENCES

Since no morphological differences were readily discerned between the Belém *tropicalis* and Cienfuegos *cubana,* a careful comparison of some of their morphological traits was undertaken. For this purpose each subspecies was grown in an uncrowded culture under optimal feeding condi-

tions at 24°–26° C. Soon after hatching the flies were transferred to shell vials containing fresh food and left there to harden for two days. Next, measurements to determine body length, wing length, costal index, 4th vein index, and 5th vein index were made on 46–50 females and 50 males of *tropicalis* and 5–6 females and 5–6 males from each of 9 strains of *cubana*. Following the convention of Burla *et al.* (1949), body length was recorded as the sum of the distances from the antenna to the base of a haltere and from the latter to the end of the anal tubercle. The number of branches of the arista was determined for females only, using 50 specimens of *tropicalis* and 7 from each of 7 strains of *cubana*.

Table 1 summarizes the data for these traits. It appears that *cubana* is a somewhat larger fly—at least the *cubana* female is larger than the *tropicalis* female, even though there is no statistically significant difference between the males of the two species—and has a slightly longer wing. The costal index is also greater in *cubana*. But the 5x index is significantly greater in *tropicalis*. This last difference may not be the property of all strains of the respective subspecies. There is probably no difference between the two subspecies with respect to 4th vein index (significantly

TABLE 1

COMPARISON OF SOME MORPHOLOGICAL TRAITS IN *D. tropicalis tropicalis* BURLA AND DA CUNHA AND *D. tropicalis cubana* SUBSP. N.*

Trait	Subspecies	N	Range	M ± m	Student's t	P
Body length (mm.)	*tropicalis* ♀	50	2.14–2.65	2.38 ± 0.016	3.17	< 0.003
	cubana ♀	54	2.20–2.75	2.46 ± 0.017		
	tropicalis ♂	50	1.88–2.38	2.12 ± 0.016	1.50	> 0.13
	cubana ♂	54	1.92–2.38	2.14 ± 0.011		
Wing length (mm.)	*tropicalis* ♀	50	1.96–2.22	2.11 ± 0.007	8.54	≪ 0.0001
	cubana ♀	54	2.00–2.35	2.22 ± 0.011		
	tropicalis ♂	50	1.73–2.06	1.90 ± 0.01	3.86	< 0.0002
	cubana ♂	54	1.76–2.06	1.95 ± 0.009		
Costal index	*tropicalis* ♀	46	1.74–2.14	1.90 ± 0.011	6.61	≪ 0.0001
	cubana ♀	54	1.77–2.28	2.03 ± 0.015		
	tropicalis ♂	50	1.64–1.89	1.76 ± 0.03	6.69	≪ 0.0001
	cubana ♂	53	1.55–2.07	1.87 ± 0.013		
4th vein index	*tropicalis* ♀	46	1.77–2.13	1.95 ± 0.013	2.00	0.04–0.05
	cubana ♀	53	1.76–2.23	1.99 ± 0.013		
	tropicalis ♂	50	1.74–2.19	1.94 ± 0.014	0.45	0.60–0.70
	cubana ♂	53	1.64–2.22	1.93 ± 0.017		
5x index	*tropicalis* ♀	46	1.50–1.99	1.68 ± 0.015	3.04	< 0.002
	cubana ♀	54	1.38–1.96	1.61 ± 0.017		
	tropicalis ♂	50	1.48–1.97	1.71 ± 0.047	0.43	0.60–0.70
	cubana ♂	53	1.46–1.97	1.70 ± 0.016		
Branches of arista	*tropicalis* ♀	50	10–12	11.12 ± 0.07	1.82	0.70–0.80
	cubana ♀	49	10–12	10.90 ± 0.10		

*N = number of flies; M = mean; m = standard error; P = probability.

different between females at the 5 per cent level but not at the 1 per cent level) or with respect to number of branches of the arista.

Although some means differ significantly, the range of values for all traits studied overlap broadly. The absolute differences between corresponding means are small, being in several instances differences in the second decimal place. It is apparent that these traits could not be used with any reliability to separate individuals belonging to the two subspecies, regardless of whether or not the flies developed under optimum conditions. No morphological differences other than those just described have yet been discovered between the subspecies when either these same strains of the subspecies or the other Greater Antilles strains of *cubana* and the Içana strain of *tropicalis* were examined.
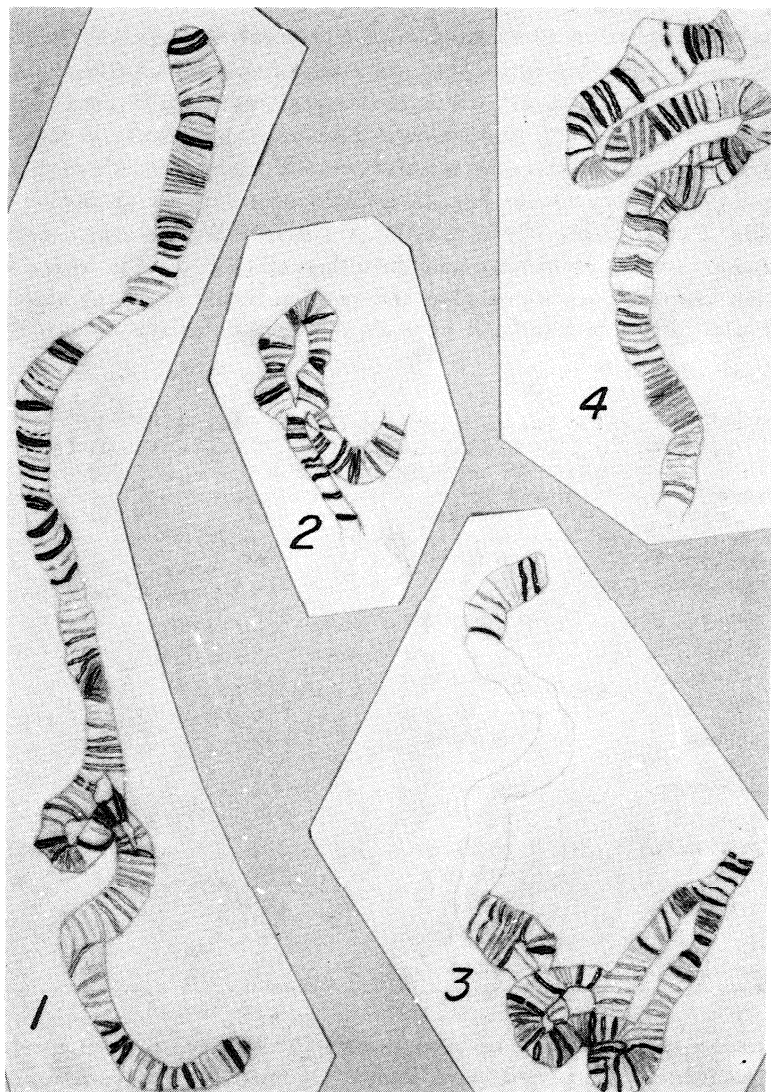
CHROMOSOMES

The metaphase plate figures in the ganglion cells of both *tropicalis* and *cubana* appear identical. The gene arrangements in the two subspecies may be compared by examining the larval salivary gland chromosomes of F_1 hybrids; inversion loops in the chromosomes of these hybrids may indicate the presence of any distinctive arrangements. Seven strains of *cubana* from Cienfuegos, five from Ciudad Trujillo, two from Río Piedras and one each from Baracoa, Constant Spring, and Port-au-Prince were intercrossed with Belém *tropicalis*. At least one *cubana* strain from each of the localities was used to obtain hybrids by both reciprocal intercrosses with the *tropicalis*. This Belém strain of *tropicalis* was homozygous for a single gene arrangement in each of its five detectable chromosome arms.

Pairing was often complete in all the chromosomes of the hybrids. In every hybrid at least two inversion loops were observed: a medium-long one in the proximal third of chromosome II L and a small inversion in the middle third of chromosome III. The former inversion is depicted singly in figures 1 and 2 and with a tandem, more basal inversion in figure 3. The inversion in chromosome III is depicted in figure 4.

Only four inversions, two in III and two in II L, have previously been reported in *tropicalis* (Burla *et al.*, 1949; Dobzhansky, Burla, and da Cunha, 1950). The inversions found in *tropicalis-cubana* hybrids do not correspond with any inversions already known.

The subbasal inversion in II L has been found heterozygous in *cubana* from wild populations of Haiti, Dominican Republic, and Puerto Rico. In fact this inversion is the only one yet discovered heterozygous in natural populations of *cubana*. It has also been observed to form a loop in Belém *tropicalis*—Jamaica *cubana* F_1 hybrids.

The fact that no heterozygous inversion was observed in the progeny of more than 50 *cubana* females taken at Soledad Central, in conjunction with the lack of heterozygotes for the II L subbasal inversion in hybrids between *cubana* from this region and Belém *tropicalis*, suggests that the *cubana* of Cuba may be, in the subbasal region of II L, homozygous for the same gene arrangement as is the Belém *tropicalis*.

FIGURES 1-4. Heterozygous inversions in hybrids between *Drosophila tropicalis tropicalis* and *Drosophila tropicalis cubana*. Figs. 1-2. A medium-long inversion in the left arm of the second chromosome. The entire chromosome arm is depicted in fig. 1. Fig. 3. Same inversion in tandem with a subbasal inversion. (An unclear region of the chromosome is represented as bandless.) Fig. 4. Distal two-thirds of the third chromosome showing a small inversion in the distal third of the chromosome.

In any event, the differences between gene arrangements in the two subspecies are relatively slight.

SEXUAL ISOLATION

In order to determine the insemination relations possible within the *willistoni* sibling group, Burla *et al.* (1949) made a number of "no choice"

experiments in which 10 females of one of these species and 10 males of
another were aged 4-6 days separately and then without etherization con-
fined together at about 25° C. in food-containing 25 × 95 mm. shell vials
(some experiments using 20 females and 20 males were made in 35 × 95 mm.
vials) for 8-12 days; then the females were dissected and their spermathe-
cae and ventral receptacles examined microscopically for spermatozoa.

Table 2 summarizes the results of a similar series of tests between a
Cienfuegos strain of *cubana* and the other sibling species and, for com-
parative purposes, includes also the results Burla *et al.* obtained using
tropicalis. From this table it appears that *tropicalis* and *cubana* differ in

TABLE 2

NUMBERS OF FEMALES DISSECTED (n) AND PERCENTAGES FOUND INSEMINATED
IN SOME EXPERIMENTS INVOLVING NO CHOICE OF MATES (DATA
MARKED WITH AN ASTERISK (*) FROM BURLA *et al.*, 1949)

Males	Females	n	% inseminated
cubana	*willistoni*	36	33.3
tropicalis	,,	112	0.0*
cubana	*paulistorum*	217	14.3
tropicalis	,,	115	27.8*
cubana	*equinoxialis*	142	0.0
tropicalis	,,	143	0.0*
cubana	*tropicalis*	63	90.5
willistoni	*cubana*	81	0.0
,,	*tropicalis*	105	3.8*
paulistorum	*cubana*	229	0.0
,,	*tropicalis*	104	65.0*
equinoxialis	*cubana*	183	0.0
,,	*tropicalis*	105	0.0*
tropicalis	*cubana*	122	68.0

that males of the latter can inseminate *willistoni* females with relatively
high frequency while *tropicalis* males are completely sexually isolated
from these females, and that *tropicalis* females frequently may be insemi-
nated by *paulistorum* males and occasionally by *willistoni* males while
sexual isolation between these males and *cubana* females is complete.

In order to determine whether these results obtained with a single strain
of *cubana* might be expected with other geographical strains, the "no
choice" experiments were repeated on a small scale using one strain of
cubana from each of the countries in which this subspecies has been col-
lected. For any particular cross not more than 20 females were recovered
and dissected. It was found that some strains differed from each other in
their insemination relations with the different sibling species. Although
insemination frequencies differed somewhat (probably not significantly in
such small samples) from strain to strain, in general the males of all these

cubana strains paralleled those of the Cienfuegos strain in possible in-
seminations. The females of some strains of *cubana* were inseminated by
equinoxialis males, a relationship shown neither by the Cienfuegos strain
nor by *tropicalis*; otherwise, the insemination relations of *cubana* females
were about the same as those of the Cienfuegos females.

Male "multiple choice" experiments were made to determine if any sex-
ual isolation exists between *tropicalis* and *cubana*. The Belém strain of
tropicalis and *cubana* strain CC-4 from Cienfuegos were used. Virgin fe-
males and males were collected and aged separately for 4-6 days; then
10 females of each subspecies—marked by clipping a slight nick in either
the right or left wing, depending upon the subspecies—and 10 males of
one subspecies were transferred without etherization to 25 × 95 mm. shell
vials containing food and left there at 25° C for one hour, the average time
required for about 50 per cent of the females to be inseminated if 10 fe-
males and 10 males of *tropicalis* are so confined together. At the expira-
tion of the time limit, the contents of the vials were etherized, the females
of the two subspecies separated, and the spermathecae and ventral recep-
tacles examined microscopically for spermatozoa.

Data obtained in the "multiple choice" experiments are summarized in
table 3. In the experiments *tropicalis* males inseminated significantly more
of their own females than of *cubana* ($\chi^2 = 25.32$; P $\ll$ 0.0001), while *cubana*
males were about equally successful in inseminating either kind of female
($\chi^2 = 0.12$; P $>$ 0.90). The Levene (1949) coefficient of isolation for *tropi-
calis* males is 0.33 ± .06, and 0.02 ± .07 for *cubana* males. The former
coefficient is significantly different from zero, but the latter is not. The
Levene joint coefficient of isolation, which measures the reproductive iso-
lation between the two subspecies, is 0.18 ± .05. The latter is significant
($t = 3.95$). It thus appears that sexual isolation may exist to a slight ex-
tent between the two subspecies due to some sexual isolation between
tropicalis males and *cubana* females—but there is none between *tropicalis*
females and *cubana* males.

When both kinds of "multiple choice" experiments are considered, it
becomes apparent that a significantly greater proportion of *tropicalis* fe-
males than of *cubana* females (69.4 and 57.1 per cent respectively) were
inseminated ($\chi^2 = 11.88$; P $<$ 0.001). The Levene coefficient of excess

TABLE 3

NUMBER OF FEMALES DISSECTED (n) AND PER CENT INSEMINATED (%) DURING
EXPERIMENTS IN WHICH MALES OF ONE SUBSPECIES HAD A CHOICE
BETWEEN FEMALES OF BOTH SUBSPECIES

♂♂	*tropicalis* ♀♀		*cubana* ♀♀		χ^2	Levene coefficient of isolation
	n	%	n	%		
tropicalis	191	73.8	189	48.7	25.32	0.33 ± .06
cubana	169	64.5	172	66.3	0.12	0.02 ± .07

insemination of *tropicalis* over *cubana* is $0.15 \pm .05$. This means that gene flow is somewhat greater from *cubana* into *tropicalis* than in the reverse direction, under conditions of the experiments.

The results of these "multiple choice" experiments may very well be due to greater sexual activity on the part of *tropicalis* females than of *cubana* females. As will be shown in the next section, this hypothesis is also supported by some evidence other than the fact that more *tropicalis* females than *cubana* females were inseminated in these experiments.

FERTILITY AND FECUNDITY

At 25° C. reciprocal intercrosses were made between Belém *tropicalis* and seven strains of *cubana* from Cienfuegos, four from Baracoa, two from the Dominican Republic, two from Puerto Rico, and one from Haiti. Five or more flies of each subspecies were used in a cross. Every cross produced progeny; but, when the F_1 flies were serially transferred to fresh food, in no case was an F_2 obtained. An intercross of Cienfuegos *cubana* and Içana *tropicalis* gave similar results.

In the same fashion as just described, five *cubana* strains, one from each country in which this subspecies has been collected, were reciprocally intercrossed with each other and with Belém *tropicalis*. All possible intercrosses (a total of 30) between these strains were made. Every cross produced an F_1. While an F_2 was easily obtained from each of the intercrosses between geographical strains of *cubana*, no F_2 could be obtained from any intercross of *tropicalis* and *cubana*. The F_1 flies from each of the 30 crosses were separated by sex, and a backcross was then attempted between each sex and each parental strain (four backcrosses for each original intercross). The F_1 males and females from intercrosses of geographical strains of *cubana* were fertile with either parental strain. On the other hand, the F_1 males from all intercrosses involving *tropicalis* were sterile no matter whether the backcross was attempted with *tropicalis* or *cubana*, while the sisters of these sterile males were fully fertile with males of either subspecies.

In order to compare the frequencies with which *tropicalis-cubana* intercrosses and backcrosses successfully produce offspring and also the relative numbers of resulting progeny, "crossability" experiments of the type made by Patterson and Dobzhansky (1945) were performed using Belém *tropicalis* and *cubana* strain CC-4 from Cienfuegos. In each of the experiments 140-170 single pairs of virgin females and males, previously aged separately for 3-5 days, were confined with food in individual creamers at 25° C; on the fifth day of confinement any creamer in which either fly had died was discarded; on the twelfth day the proportion of remaining creamers in which progeny were evident was recorded. Of each of the different types of crosses attempted, the first 50 creamers recorded as having housed a fertile pair were tagged in order that the emerging progeny could later be counted as a measure of fecundity. (In all, seven such creamers subsequently had to be discarded for technical reasons.)

The data on fertility and fecundity as measured in these experiments are summarized in table 4. In this table *tropicalis* is symbolized by T and *cubana* by C, with the female parent being given first. F_1 hybrids are represented TC or CT according to whether the female parent was *tropicalis* or *cubana*, respectively.

The fertility data in table 4 show that once again all intercrosses between *tropicalis* and *cubana* produced fertile hybrid females and completely sterile hybrid males. It should also be noted that the *tropicalis* control crosses were more successful (82.6 ± 3.9 per cent fertile) than the *cubana*

TABLE 4

NUMBER OF PAIR MATINGS (n) SCORED AND PER CENT FERTILE, ALSO NUMBER OF CULTURES IN WHICH THE OFFSPRING WERE COUNTED (N) AND MEAN NUMBER OF OFFSPRING PER FERTILE MATING (M). *Drosophila tropicalis tropicalis* ABBREVIATED T; *Drosophila tropicalis cubana*, C. FEMALE PARENT SHOWN FIRST.

	Matings	N	% fertile	n	M ± m
Controls	T × T	92	82.6 ± 3.9	50	44.0 ± 3.4
	C × C	79	63.3 ± 5.3	44	44.9 ± 4.7
P_1	T × C	103	71.8 ± 4.4	50	49.4 ± 3.9
	C × T	101	70.3 ± 4.5	50	53.1 ± 3.7
Backcrosses	TC × T	129	72.9 ± 3.9	50	81.2 ± 3.4
	CT × T	110	80.9 ± 3.7	49	74.2 ± 3.5
	TC × C	88	88.6 ± 3.4	50	80.0 ± 3.9
	CT × C	120	91.7 ± 2.5	50	78.4 ± 3.5
	T × TC	82	sterile		
	T × CT	138	″		
	C × TC	104	″		
	C × CT	138	″		

ones (63.3 ± 5.3 per cent fertile). The frequencies of fertile intercrosses were intermediate (70.3 per cent and 71.8 per cent) between the comparable control frequencies. For the backcrosses of hybrid females the range varied from a frequency (72.9 per cent) about the same as for the intercrosses to frequencies (88.6 per cent and 91.7 per cent) in excess of that for the *tropicalis* controls.

In all successful kinds of crosses involving *tropicalis* males the frequencies of fertile matings (70.3–82.6 per cent) with the four different kinds of females did not differ significantly ($\chi^2 = 6.18$; $P > 0.10$). This may be interpreted to mean that the different kinds of females were on the average equal in fertility. However, when the different kinds of crosses involving *cubana* males are compared, it is seen that the frequencies of fertile matings (63.3–91.7 per cent) did differ significantly ($\chi^2 = 32.38$; $P \ll 0.0001$). If this was not due to differences in fertility of the females, then it appears likely that the success with which *cubana* males mate is very much dependent upon the genotype of the females—hybrid females, as well as *tropicalis* females, probably being more active sexually than are *cubana*

females. If this is true, mating success of *tropicalis* males is less dependent upon the relative level of sexual activity of the females than that of the males themselves when an excess of time (more than 5 days) is allowed for mating.

The absence of progeny from crosses attempted with hybrid males was not due to failure of these males to copulate. On the contrary, hybrid males mated rather readily with parental type or hybrid females, but transferred no spermatozoa. In fact, hybrid males do not produce any motile spermatozoa. Microscopic examination of the testes of hybrid males disclosed bundles of degenerating spermatids throughout most of the gland and only degeneration masses in the proximal region. Meiosis was not studied.

The mean numbers of offspring produced in the control crosses and different kinds of *tropicalis-cubana* intercrosses and backcrosses are presented in the last column of table 4. The means for the controls and intercrosses were about equal (44.0–53.1), as were those for backcrosses of hybrid females (74.2–81.2); however, the latter were significantly greater than the former. This means that the hybrid females were heterotic—the heterosis being expressed as increased fecundity.

DISCUSSION

Of the seven sets of Drosophila subspecies listed by Patterson and Stone (1952) *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* in the nature of their relationships (Patterson and Dobzhansky, 1945) most closely resemble *D. tropicalis tropicalis* and *D. tropicalis cubana*. In both cases the subspecies are easily intercrossed to produce viable hybrids; the F_1 male hybrids are completely sterile and have degenerating spermatids in their testes; sexual isolation, if any, between the subspecies is slight and confined to one of the reciprocal intercrosses; and there is relatively little chromosomal reorganization. Two distinctive inversions have developed between *tropicalis* and *cubana*, but only one long inversion between *pallidipennis* and *centralis*. The extent of chromosomal reorganization between the latter two subspecies remains therefore the least yet known between two such forms.

Although female hybrids between *pallidipennis* and *centralis* are generally somewhat less fertile than females of either subspecies, the fertility of female hybrids between *tropicalis* and *cubana* does not differ significantly from that of either parental type of female. Furthermore, the increased fecundity of *tropicalis-cubana* female hybrids shows them to be heterotic. Heterotic subspecific hybrids are not unique; Wharton (1944) found that hybrids between *D. mercatorum mercatorum* and *D. mercatorum pararepleta* exceeded their parents in vigor and fertility. Since *tropicalis-cubana* hybrid females are no less fertile than females of either subspecies, the increased fecundity of the hybrids would offset to a considerable extent the partial block that male hybrid sterility presents to gene exchange should these subspecies meet and hybridize in nature. Nonetheless, steril-

ity of the hybrid males is the only well-developed reproductive isolating mechanism possessed by these subspecies.

The northern-most distribution reported for *tropicalis* is in the Territory of Rio Branco, Brazil (Burla *et al.*, 1949); the southern-most distribution now known for *cubana* is in Jamaica. Although collecting in the Lesser Antilles would be expected to extend the distribution of one or both of these forms, it appears certain that the extent of reproductive isolation between them is insufficient to permit maintenance of distinctive sympatric populations; therefore, divergence to the level of full species has not yet occurred. Nevertheless, the differences already described, when taken in conjunction with geographic replacement, are quite adequate for ranking the forms as distinct subspecies.

The ranking of *tropicalis* and *cubana* as subspecies is independent of the fact that these forms are only known to differ morphologically in the mean measurements of certain characters that overlap to such an extent as to be completely useless for classifying individuals; in contrast to this, Patterson and Dobzhansky (1945) were rather easily able to separate *pallidipennis* and *centralis* grown under uniform conditions, and also managed to separate about fifty per cent of the individuals that had not been grown under uniform conditions. Morphological differences between *tropicalis* and *cubana* are even more cryptic than those found between the other sibling subspecies: *americana* and *texana* (Patterson, Stone, and Griffen, 1940); *meridiana* and *rioensis* (Patterson, 1943); *mercatorum* and *pararepleta* (Wharton, 1944); and *fulvimacula* and *flavorepleta* (Patterson, 1952).

Notice should be taken of the fact that *tropicalis* and *cubana* belong to the *willistoni* sibling species group which includes three species other than these subspecies. The morphological differences between the sibling species are so slight that it is very nearly impossible to separate on such a basis the females of three species. Spassky and Frota-Pessoa (personal communication) are able to classify the males of some strains of all four species by means of rather subtle differences between the externally visible genitalia. Still, the greatest morphological differences known between the four species are between the ventral receptacles and extent of chitinization of the spermathecae of the *D. tropicalis* subspecies and those of the other three species. While *tropicalis* and *cubana* have diverged genetically, they have not abandoned the morphological conservatism characteristic of the sibling group to which they belong.

Although morphologically *tropicalis* and *cubana* have diverged only to a nearly imperceptible extent, they show considerable differences in general level of adaptedness as reflected by their frequencies relative to the other siblings sympatric with them. Dobzhansky and Pavan (1950) have shown that in Brazil the *willistoni* sibling group is dominant in most collections of Drosophila. Da Cunha *et al.* (1950) found that usually the most widespread of the sibling species, *D. willistoni*, is also the most abundant. According to personal communication from Dobzhansky and da Cunha the only locality in which they have found *tropicalis* to be dominant is in

Marajó Island, an island previously reported by da Cunha *et al.* to be eco-
logically unfavorable for the *willistoni* siblings. Ordinarily *tropicalis* was
outnumbered about 50 to 1 by the other siblings in the Brazilian collec-
tions. In the Greater Antilles, the *willistoni* siblings made up about 9 per
cent to about 36 per cent of the total samples (from Cienfuegos and Port-
au-Prince, respectively) but were only second to fourth in abundance. The
best data on relative frequencies of the different siblings are available for
Cienfuegos and Ciudad Trujillo. In the former locality *cubana* outnumbered
willistoni almost 2 to 1 and was about equally as frequent in the latter.
As much as a third of the *willistoni* sibling sample (in Río Piedras) was *D.
equinoxialis*. These relations make it apparent that *cubana* is better adapted
to compete with the species most closely related to it than is *tropicalis*.

SUMMARY

A new subspecies, *Drosophila tropicalis cubana*, belonging to the sib-
lings of the *willistoni* group of the subgenus Sophophora and distributed
throughout the Greater Antilles is described and compared with the South
American subspecies, *Drosophila tropicalis tropicalis* Burla and da Cunha.
The two subspecies overlap widely in the ranges of values for certain mor-
phological traits conventionally used in describing species of Drosophila;
although some of the means differ significantly, not one of the traits can
be used for separating the subspecies with any degree of reliability. There-
fore, the morphological differences are cryptic. Sexual isolation between
the subspecies is minor, if it exists at all, and confined to one of the re-
ciprocal intercrosses, both of which produce viable hybrids—fertile fe-
males that are heterotic as shown by increased fecundity and completely
sterile males that have testes containing degenerating spermatids. At
present the subspecies are best distinguished by intercrossing with known
strains and examining the fertility of the F_1 hybrids. The gene arrange-
ments of the subspecies differ only slightly, by a medium-long inversion
in II L and a very small one in III. The northern subspecies is generally

better adapted to compete with the closely related siblings than is the South American subspecies. There are some differences between the sexual isolation of each of the subspecies from the other siblings, but these may be strain rather than subspecific differences.

Subspeciation of *D. tropicalis* is compared with that of other Drosophila, especially with *D. pallidipennis* subspeciation which it most nearly resembles.

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